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ETHYLENE PRODUCTION BY PLANTS IN A CLOSED ENVIRONMENT

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ABSTRACT

Ethylene production by 20-m^2 stands of wheat, soybean, lettuce and potato was monitored throughout growth and development in NASA's Controlled Ecological Life Support System (CELSS) Biomass Production Chamber. Chamber ethylene concentrations rose during periods of rapid growth for all four species, reaching 120 parts per billion (ppb) for wheat, 60 ppb for soybean, and 40 to 50 ppb for lettuce and potato. Following this, ethylene concentrations declined during seed fill and maturation (wheat and soybean), or remained relatively constant (potato). Lettuce plants were harvested during rapid growth and peak ethylene production. The highest ethylene production rates (unadjusted for chamber leakage) ranged from 0.04 to 0.06 ml m^{-2} day^{-1} during rapid growth of lettuce and wheat stands, or approximately 0.8 to 1.1 nl g^{-1} fresh weight h^{-1} Results suggest that ethylene production by plants is a normal event coupled to periods of rapid metabolic activity, and that ethylene removal or control measures should be considered for growing crops in a tightly closed CELSS.

INTRODUCTION

As part of studying plants for life support applications in space, we have grown crops in a large, closed chamber at NASA's Kennedy Space Center /1/. The chamber is a retired hypobaric test vessel used during the Mercury Project in the 1960s that has since been fitted with electric lamps, air circulation ducts and fans, heat exchange systems, and hydroponic nutrient delivery systems to support a 20 m^2 crop growing area. Because the chamber can be tightly closed, gas exchange rates (e.g., photosynthesis, respiration, and transpiration) of the crop communities can be tracked throughout growth and development /2/. The tight closure also allows tracking of trace volatiles and contaminants arising from the crop stands and/or materials inside the chamber. Build-up of ethylene gas in the chamber has been a particular concern because of plants' tendencies to produce ethylene /3,4/. Ethylene is known to control or influence a wide range of growth and development processes in plants, including stem swelling, seedling hook opening, leaf epinasty, floral abortion, leaf abscision, and fruit ripening /3,4/. For some responses, (e.g., inhibition of flower opening of some rose cultivars), ethylene concentrations as low as 4 parts per billion (ppb) can be effective /3/, while most other responses are typically seen between 0.1 to 10 parts per million (ppm). We report here on ethylene measurements made throughout growth and development of four higher plant species selected for CELSS studies.

MATERIALS AND METHODS

All studies were conducted in NASA's Biomass Production Chamber (BPC) located at Kennedy Space Center, FL, USA. Plant species included wheat (*Triticum aestivum* L. cv. Yecora Rojo), soybean (*Glycine max* [Merr.] L. cv. McCall), lettuce (*Lactuca sativa* L. cv. Waldmann's Green), and potato (*Solanum tuberosum* L. cv. Norland). For each study, the entire 20 m^2 area of the chamber was planted with one species. Plants were grown hydroponically using recirculating nuutrient film technique /5/, with lighting provided by 96, 400-W high-pressure sodium lamps. Carbon dioxide (CO_2) concentrations during the light cycles were maintained at at 1000 ppm (0.10 kPa), and temperatures and photoperiods were controlled to optimize growth for each species /6,7/.

Chamber leakage during periods of closure was determined by adding CO_2 to the chamber before planting and tracking the concentration decay over time /8/. Leakage rates determined in this manner averaged 10% of the volume per day /9/. Typically the chamber was entered once daily for horticultural and maintenance activities, which would increase the leakage rate, although the precise effects of chamber entrance episodes on leakage was not determined. Hence all ethylene concentration and production estimates should be considered conservative.

Air samples from the chamber were analyzed for ethylene with Photovac 10S70 and Photovac 10S Plus portable, gas chromatographs (GC) with photoionization detector (PID) at 11 eV. The GC/PID was set to automatically sample the air via 3.2 mm o.d. X 76 cm Teflon® lines. One ml of air sample was automatically injected from the sample loop onto the GC/PID column. Four different columns were used at various times during the different studies: 1) 183 cm X 3.2 mm 6.6% CSP-20M on Carbopak B and 183 cm X 3.2 mm Carbopak BHT 100 in tandem with 15-cm precolumn; 2) 244 cm X 3.2 mm XE-60 on Carbopak B with 15-cm precolumn; 3) 900 cm X 0.53 mm KCL/Alumina fused silica with precolumn; 4) 900 cm X 0.53 mm CP-Sil™5CB with precolumn. The detector gain was set at 1,000 for greatest sensitivity and the isothermal oven was set at 30°C. Ultra-pure air carrier gas was set at 15 ml min^{-1}. Detectable limits of 2 ppb were calculated. Replicate injections of ethylene standards showed variability of less than 1% and a standard deviation of 0.27 ppb. The presence of ethylene was verified by changing the column, injecting the standard and sample, and comparing retention times.

RESULTS AND DISCUSSION

A composite graph of chamber ethylene levels over time for the different species is shown in Fig. 1 A. Ethylene concentrations varied among species, reaching maximum levels of 120 ppb with wheat, 60 ppb with soybean, and 40 to 50 ppb for lettuce and potato. The highest concentration of ethylene during wheat growth occurred near 28 days, during rapid vegetative growth and nearly 10 days before heading and anthesis. The highest rates of stand photosynthesis, respiration, and nutrient uptake also occurred near this time /6/. Ethylene concentrations during soybean growth peaked near 40 days, during rapid growth, declined during pod filling, and then increased slightly just prior to harvest. Flowering of the McCall soybean plants was first apparent near 30 days and continued until about 70 to 75 days. Ethylene concentrations during lettuce growth showed a rapid increase between 25 and 28 days, afterwhich plants were harvested. (Note, lettuce was only grown for its vegetative biomass and did not reach flowering and senescence). Ethylene concentrations during potato growth rose gradually until about 50 days and then remained relative constant. Tubers first appeared on potato plants near 30 to 35 days and then continued to enlarge throughout growth.

For all tests, atmospheric measurements made prior to each planting and during early growth of each species showed low or undetectable levels ethylene, indicating that construction materials and internal components of the chamber (e.g., plastics, paints and caulking compounds) were not a significant source of ethylene. Thus the ethylene observed in the crop growth studies was biogenic in origin.

Except for the lettuce study, ethylene concentrations in the chamber did not continue to increase over time, suggesting that chamber leakage outstripped plant production during later stages of growth. It is possible that ethylene was metabolized over time by the plants, or by microbes in the nutrient solution systems, but there little evidence for significant amounts of ethylene catabolism by plants /10,11/. Nutrient solution microflora showed relatively little change in species composition and total counts after 1 to 2 weeks after planting and root establishment /12/, suggesting that microbial effects, if any, should have been constant over time.

By determining the standing biomass over time from the photosynthetic uptake of CO_2 (Fig. 1 B) /7/ and dividing by the ethylene concentrations, the relative level of ethylene produced per unit biomass can be estimated (Fig. 1 C). The patterns for wheat, soybean, and potato suggest that the highest rates of

production occurred when the plants were young and ethylene were first detected. Normalized data from lettuce studies show no clear trend, although data were limited because of the short cropping cycle for lettuce and the relatively low biomass. Because ethylene is often associated with ripening and senescence events, the high ethylene production when plants were young was somewhat surprising; yet the trends are consistent with findings by Roberts and Osborne /13/ using single plants and suggest the ethylene production is normal during healthy vegetative growth.

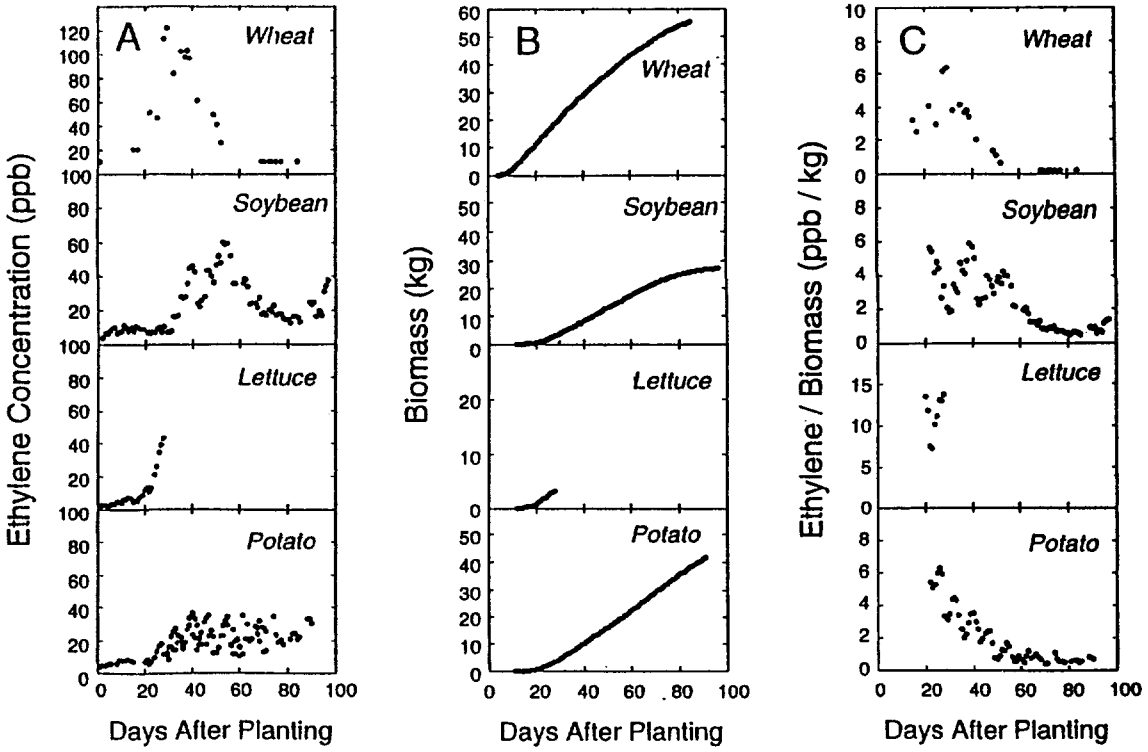

Fig. 1. (A) Ethylene concentration inside NASA's Biomass Production Chamber during studies with wheat, soybean, lettuce, and potato. (B) Standing dry biomass of crops grown in the Biomass Production Chamber. (C) Ethylene concentrations normalized for stand biomass of crops.

Because of chamber leakage and periodic entrances during the studies, estimation of absolute ethylene prodution rates by the plants is difficult. However, by determining slopes during periods of ethylene increase, rates of production during vegetative growth of wheat and lettuce stands would have at least been 0.04-0.06 ml m^{-2} day^{-1}, which would equate to 15 nl g^{-1} DW h^{-1} for lettuce and 7-8 nl g^{-1} DW h^{-1} for wheat (Fig. 1 A).

Could ethylene be a problem for plants grown in tightly closed systems for life support in space? Young potato leaves (especially leaflets) in our studies occasionally showed epinasty, while wheat leaves showed longitudinal, epinastic rollling, both of which may have been caused by ethylene. Chronic exposures to ethylene levels of 50 to 100 ppb have been shown to reduce growth of lettuce and easter lilies /14,15/, suggesting levels in our studies were potentially injurious. At the production rates noted for wheat during rapid growth, maximum levels in a simlar system but with an order of magnitude of less leakage could be expected to exceed 1.0 ppm. Moreover, this does not include complications that might arise if ethylene autocatalysis occurs, as has been shown in ripening fruit tissues /3,4/. In more recent tests, permaganate filters were effective for controlling levels to 40 ppb or lower in our system, and this likely could be improved by increasing amount of permanganate or its exposure to the air. However, this

would be a consumable that would have to be imported to a CELSS. Tests with continuous operating systems such as catalytic burners, selective membranes, and/or ethylene metabolizing microbes might also be explored for ethylene control in a CELSS. A question then arises as to whether a small amount of ethylene in the atmosphere may be beneficial during certain phases of plant growth and development. If this is the case, ethylene levels might then be managed similar to other environmental factors to optimize plant development and yield.

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